A COMPUTER-ASSISTED SYSTEM (3D-DIAS) FOR RECONSTRUCTING AND QUANTITATIVELY ANALYZING THE DYNAMIC 3D RELATIONSHIPS OF THE OUTER SURFACE, NUCLEUS, PSEUDOPODS AND VESICLES OF A CRAWLING CELL

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Cell motility is fundamental to animal development, cellular immunity, maintenance of animal tissues in adult organisms, metastasis, disease progression and the survival of most single cell organisms. For that reason, understanding how cells locomote represents a pervasive theme in biology and medicine. Because most living cells are viewed through conventional microscopes from above or below, we tend to interpret both their cellular and intracellular behavior in two dimensions. However, static electron micrographs and confocal 3D reconstructions of fixed cells have demonstrated that they are indeed very three dimensional. Therefore, understanding how cells locomote must include 3D descriptions of behavior and dynamic architecture. To accomplish this, we have developed a computer-assisted 3D dynamic image analysis system, 3D-DIAS, that reconstructs the surface, nucleus and pseudopods of living crawling cells, and that will track vesicle behavior in 3D. Using either differential interference contrast microscopy to visualize the cell surface, nuclear membrane, and pseudopodial regions, or, a Noran Oz near-real time laser scanning confocal microscope to visualize vesicles in living cells, 30 optical sections are obtained in a one second period through the z-axis of a cell, and this procedure is repeated every second. The optical sections are digitized into the 3D-DIAS program, which image-processes each section, automatically outlines the edges of the cell by a pixel complexity measurement, and stacks outlines. In the case of the nucleus and pseudopods, edges are manually entered. In the case of vesicles, 3D-DIAS automatically interprets position. 3D surfaces are encapsulated by faceting algorithms, and the final 3D image of the living cell, nucleus, pseudopods and vesicles can be viewed dynamically at any angle through a 3D stereoworkstation. Over 100 parameters of motility and dynamic morphology are generated every second by 3D-DIAS for the cell surface, nucleus, and pseudopods, and over 20 parameters of motility are generated every second for vesicles, providing the first high resolution, quantitative description of cellular and intracellular motility in 3D. This system has been used to identify behavioral defects in cytoskeletal and signal transduction mutants, HIV-infected T cells and HIV-induced T cell syncytia, normal and neoplastic cells, developing cardiac and embryonic cells, and embryonic organs. The audience will be supplied with 3D red and blue glasses, and provided with a computer-assisted demonstration of 3D reconstruction, and the 3D dynamics of the cell surface, nucleus, F-actin enriched pseudopods and vesicles of live, crawling Dictyostelium amoebae searching for and responding to chemotactic gradients of the attractant cAMP. Development of a quantitative 4D confocal system, a near real-time 3D reconstruction/motion analysis system, a high speed 3D system (performing complete 3D reconstruction every 0.1 sec) and a virtual reality system will also be briefly described.